Selecting vegetative/generative/dwarfing rootstocks for improving fruit yield and quality in water stressed sweet peppers

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ABSTRACT

Rootstock breeding for vegetable crops includes desirable traits such as compatibility with the scion, increased productivity and quality under stressful environments and improved use of soil, water and fertilizer resources. The effects of three commercial rootstocks (Atlante, Creonte and Terrano) on the agronomical and physiological responses of a commercial sweet pepper variety (cv Herminio) to deficit irrigation (50% of optimal) have been studied. Although the three rootstocks increased total and marketable yield under control and deficit irrigation, Creonte produced the most productive and water use efficient plants, with until 25% more marketable yield than the ungrafted cv Herminio, and about 10% more than the other rootstocks, although in detriment of some chemical fruit quality traits. Moreover, the plants grafted onto Creonte registered the highest photosynthetic activity and leaf water content and more stable leaf area and biomass under water stress, while those parameters were more reduced in the other graft combinations. These Creonte-mediated effects were not related to root biomass (since it was more affected by the stress in this rootstock) but rather to the capacity of maintaining a high reproductive/vegetative ratio, while Atlante is a vigorous vegetative rootstock and Terrano is rather a dwarfing-reproductive rootstock that produces efficient compact plants without negative effects on fruit quality.

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1. Introduction

The majority of climate change scenarios predict an increase in drought incidence throughout different regions of the world (IPCC, 2007). Because agricultural activities are water intensive, the increase in arid and semi-arid cropland, along with increases in population, will produce greater water demands and exploitation that will directly affect crop growth, survival and yield (Chaves et al., 2009). The effects of water stress can be direct: such as decreased CO2 availability caused by diffusion limitations through the stomata and/or the mesophyll (Flexas et al., 2007); or by alteration in CO2 fixation reactions (Lawlor and Cornic, 2002). Photosynthetic responses to water stress are complex since they involve the interplay of limitations taking place at different parts of the plant (Chaves et al., 2009). The limitation of plant growth due to low water availability is mainly due to reductions of plant carbon balance, which is largely dependent on photosynthesis (Flexas et al., 2009). One possible solution to reduce yield losses and improve crop growth under water-deficit conditions involves the use of grafts using rootstocks capable of palliating the effects of this stress in the shoot (Schwarz et al., 2010; Albacete et al., 2015).

Currently, the cultivated area of grafted Solanaceae and Cucurbitaceae has increased tremendously in recent years because the objective of grafting has been greatly expanded (Lee et al., 2010). Zones that produce grafted horticultural products of great economic importance, have in recent years begun to use grafted plants to enhance growth and yield (Lee et al., 2010), tolerance to abiotic stress as low temperature (Schwarz et al., 2010; Venema et al., 2008), uptake of nutrients (Colla et al., 2010a; Savvas et al., 2010), water-use efficiency (Rouphael et al., 2008a; Cantero-Navarro et al., 2016), reliance on susceptible cultivars to meet specific market demands (Sakata et al., 2007), increase synthesis of endogenous hormones (Dong et al., 2008), improve alkalinity tolerance (Colla et al., 2010b), reduce uptake of persistent organic pollutants from
agricultural soils (Otani and Seike, 2006, 2007), raise salt and flooding tolerance (Fernández-García et al., 2004a, 2004b; Yetisir et al., 2006; Martínez-Rodríguez et al., 2008; He et al., 2009), limit the negative effect of boron, copper, cadmium, and manganese toxicity (Edelstein et al., 2003; Rouphael et al., 2008b; Arao et al., 2008; Savvas et al., 2009) and get better photosynthetic rate (Davis et al., 2008; He et al., 2009).

Generally, drought reduces not only nutrient uptake by the root but also nutrient transport from the root to the shoot due to a restricted transpiration rate, depressed active transport, and reduced membrane permeability. On the other hand, several conflicting reports exist on the quality of fruit vegetables influenced by grafting, and whether the effects of grafting are advantageous or deleterious (Flores et al., 2010; Proietti et al., 2008; Rouphael et al., 2010). The differences in reported results may be attributable in part to different production environments and agricultural practices, type of rootstock/scion combinations used, and harvest date. In addition, very little attention has been paid to how the use of different rootstocks can affect fruit quality in grafted sweet pepper (Colla et al., 2008). Through the rootstock, water management can affect the synthesis of phytochemicals. Generally, a reduced water supply increases the contents of phytochemicals, such as phenolic compounds and anthocyanins (Dixon and Paiva, 1995). In this sense, deficit irrigation and grafting strategies are relatively new tools for managing plant growth and improving fruit quality (Sánchez-Rodríguez et al., 2012).

It is well known that seed market provides a collection of rootstocks for vegetable crops with different properties that make them more or less suitable for specific ‘G×E’ combinations, where ‘C’ is the genotype of the variety to be grafted and ‘E’ the particular environment where the crop will develop. ‘E’ includes soil/rootzone properties, cycle duration, climatic conditions, pathogens, water quality and quantity, etc. Gaining insights in the physiologico/agronomical traits of the available rootstocks (commercial or pre-breeding) will certainly contribute to a better selection of the adequate rootstocks for each agro-environment and to further development of new improved rootstocks able to increase crop productivity, resilience, resource use efficiency, yield stability and quality. For example, vigorous vegetative rootstocks are more adequate for large fruited tomato varieties growing in long cropping cycles, while generative rootstocks that put more energy into reproductive than into vegetative structures are more adequate for small fruited varieties cultivated at any cropping cycle or for large fruited ones growing in short cropping cycles. The aim of this work was to study the behaviour of three commercial pepper rootstocks on vegetative/reproductive balance, gas-exchange and fruit yield and quality of grafted sweet pepper grown in greenhouse under standard and deficit irrigation regimes. This knowledge enables choices to be made about which rootstock is better to cope with abiotic challenges such as low water availability.

2. Materials and methods

2.1. Plant material and greenhouse conditions

Using the procedure of Japanese top graft procedure, the sweet pepper cultivar ‘Herminio’ F1 (Syngenta Seeds, USA) was grafted onto three commercial rootstocks: Atlante (Ramiro Arnedo, Spain), Terrano (Syngenta Seeds, USA) and Creonte (De Ruiter, Monsanto Seeds, Holland). Ungrafted ‘Herminio’ plants were used as control. Grafted and ungrafted plants were transplanted on 5th January to an unheated arch-shaped multispan greenhouse covered with thermal polyethylene, located at the ‘Torreblanca’ Experimental Farm of IMIDA in Murcia, SE Spain (lat. 37° 45’ N, long. 0° 59’ W). The original soil type was clay loam soil, pH 7.7 and the electrical conductivity of saturated soil extract 5.47 dS m⁻¹. Prior to transplanting (10th August), the soil was biosolarized by adding a mixture of 5 kg m⁻² of sheep and chicken manure (2:1, w/w) and covering the soil with transparent, low-density plastic film (50 μm) for 90 days. Plants were grown in single rows 100 cm apart with 40 cm between each plant in a given row (25,000 plants/ha plant density). The field trial was conducted following the cultural practices commonly used in commercial sweet pepper production in this area. The crop cycle ended on 16th of August 2011 after six harvests. The control treatment (no stress: NS) received 100% irrigation requirements (being based on estimations of the weekly crop evapotranspiration, ETo), whereas moderate water stress treatment (stress: S) corresponded to 50% of the amount of the control treatment. The experimental design was a randomized block design with four replications for drip irrigation. They began 181 days after transplant and were maintained during 30 days. Total water applied in Non-Stress was 618.55 L m⁻² and in stress was 506.04 L m⁻².

The treatment was watered with a nutrient with the following composition (g m⁻³): N, 30.3; P2O5, 28; MgO, 10.36; K2O, 54; CaO, 27.2. Throughout the crop cycle. The air temperature inside the greenhouse was monitored during the growing cycle using a Hobo U12 temperature data logger (Onset, Massachusetts, USA), respectively.

2.2. Plant growth measurements

Fifteen grafted and ungrafted plants per greenhouse unit were used for measuring different parameters individually. Plant height, stem diameter, leaf number and area and total leaf, stem and root fresh (FW) and dry (DW) weights were measured at the end of the crop (1st August 2012). Leaf area was measured using a leaf area meter (LI-COR-3100C; LI-COR Inc., Lincoln, Nebraska, USA), and the root biomass was extracted from a similar soil volume 40 × 40 × 40 cm³ in all plants. DW were determined by drying each separate plant material in an oven at 60 °C until constant weight. Since the differences observed in aerial biomass between genotypes were similar on both FW and DW basis, only FW data are presented.

2.3. Gas exchange measurements

Gas-exchange and chlorophyll fluorescence were monitored in fully expanded leaves in the generative plant stage. The closest leaf to the recently set fruit was used to measure both parameters. Measurements were carried out 20 days after fruit set (205 DAT), from 9:00 am to 11:00 am (GMT). Net CO₂ assimilation rate (Amax, mmol CO₂ m⁻² s⁻¹), stomatal conductance (gs, mmol m⁻² s⁻¹), transpiration rate (E, mmol H₂O m⁻² s⁻¹), substomatal CO₂ concentration (Ci, μmol CO₂ mol⁻¹ air) and water efficiency, were measured in steady-state conditions under conditions of saturating light (800 μmol m⁻² s⁻¹ and 400 ppm CO₂) with a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA).

2.4. Fruit yield and quality

Sweet pepper fruits were harvested from the above-mentioned 15 plants used for growth measurement. Quality commercial production was evaluated according to commercial practices, as were discarded fruits with physiological disorders (sunscald, BER, etc.) that were unmarketable. Twelve randomized fruits (4 per replication of the plant material) per treatment were selected at 196 DAT in order to measure fruit quality, which included soluble solid content (°Brix), titratable acid, vitamin C, total phenolics content and antioxidant capacity.

The total soluble solids content (°Brix) was determined using a refractometer (Reichert Analytical Instruments, Depew, New
York, USA). Briefly, fruit samples were blended and centrifuged at 9000 x g for 25 min at 4 °C. The soluble solids in the supernatant were then determined. A sample of the same supernatant was also used to measure titratable acidity. For this, 6 ml of supernatant were mixed with 50 ml deionised water. The titratable acidity was determined by an automatic titrator (Titroline easy, Schott, Mainz, Germany). Citric acid was used as reference for the calculations. The content of vitamin C, measured as ascorbic acid (AA) and dehydroascorbic acid, was measured in fresh mesocarp tissue using high-performance liquid chromatography (Shimadzu Corporation, Canby, OR) equipped with a degasser, DGU-20A, autosampler SIL-30AC, column oven CTO–10AS, communications module CMB–20A, and diode array detector SPD–20 (Rodríguez-Hidalgo et al., 2010) The total phenolics content and anti-oxidant activity were analysed according to Lara et al. (2011). Briefly, 0.5 g of each sample was homogenised in 3.0 ml 100% (v/v) methanol and centrifuged at 1200 x g for 15 min at 4 °C (Heraeus Fresco 21; Thermo Scientific, Osterode, Germany). The total phenolics content was determined by the Folin–Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965). A 0.1 ml aliquot of the supernatant was mixed with 0.15 ml of Folin–Ciocalteu reagent and 1.0 ml 4 g l−1 NaOH/20 g l−1 Na2CO3. The absorption of the solution was measured at 750 nm in a spectrophotometer (SmartSpecTM Plus; Bio Rad Laboratories, Inc., Hercules, California, USA). Each measurement was compared with a standard curve of chlorogenic acid and expressed as mg chlorogenic acid equivalent (CAE) Kg−1 FW. The anti-oxidant activity was evaluated in terms of free radical-scavenging capacity. A fresh solution of 0.7 mM 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical in 100% (v/v) methanol was prepared each day. A 0.1 ml aliquot of the supernatant was added to 0.9 ml of DPPH stock solution. The absorption of each sample was measured at 515 nm in a spectrophotometer against a blank of 100% (v/v) methanol. Measurements were compared with a standard curve of ascorbic acid concentrations and expressed as mg ascorbic acid equivalent anti-oxidant capacity (AAE) Kg−1 FW.

2.5. Experimental design and statistical analysis

The experimental design was a randomized block design. Each treatment (plant material) had three blocks and five plants each. The Statgraphics statistical package was used to calculate significant differences by ANOVA and means were compared at a probability of P ≤ 0.05 according to LSD test.

3. Results

3.1. Gas-exchange parameters

Plants grafted onto Creonte rootstocks registered the highest photosynthetic rates under control and water stress conditions compared to Herminio ungrafted and grafted onto Terrano (Fig. 1a). Although water stress reduced photosynthesis by 12% (Atlante and Creonte) and 22% (non-grafted Herminio and Terrano), only the plants grafted onto Creonte registered significant higher values compared to the non-grafted Herminio plants. Similar results were obtained for stomatal conductance, transpiration and mesophyll internal CO2 concentrations (Fig. 1b–d). Curiously, the plants grafted onto Terrano showed a particular behaviour in gas-exchange parameters, since they registered 33, 69, 32, 61% less A, gs, Ci and E than the non-grafted Herminio plants under well-watered and water stress conditions, respectively (Fig. 1a–d). The intrinsic water use efficiency (WUEi = A/Ep) was similar in all genotypes, but the plants grafted onto Terrano registered a 35% increase under water stress (Fig. 1e). Although water stress reduced chlorophyll fluorescence (Fv/Fm) in all plants, no differences were found between graft combinations (data not shown).

3.2. Plant growth related parameters

Plants grafted onto Atlante produced the highest shoot, leaf and stem vegetative biomass under non-stress conditions, but it was reduced by water stress until values similar to Herminio ungrafted and grafted onto Creonte (Fig. 2a–d). Plants grafted onto Terrano registered the lowest shoot biomass under water stress. Plants grafted onto Atlante and Creonte had 15% bigger roots under control conditions compared to non-grafted Herminio and Terrano, but the differences were not significant (Fig. 2d). Water stress only reduced the root biomass in Creonte (35%) and Atlante (10%), although the root biomass of the later was 25% higher than the other rootstocks under water deficit.

Atlante produced the tallest plants under both growing conditions and Terrano the lowest ones (50% than Atlante), but the water stress did not affect this parameter (Fig. 3a). However, these differences in plant height between Atlante and Terrano graft combinations were not observed in total leaf number or leaf area (Fig. 3b, c), registering similar values to the other plants under both growing conditions. Leaf water content, expressed as FW/DW ratio, was also reduced by water deficit in all plants, but it remained more elevated in plants grafted onto Atlante and Creonte (Fig. 3d).

In general, water stress reduced those shoot growth-related parameters in all the genotypes and more significantly in Atlante and Terrano grafted plants. Nevertheless, plants grafted onto Creonte registered the highest growth stability and values in those parameters under water stress, although only significant differences were found in shoot biomass and leaf area (20–30% higher) when compared to Terrano. Since Terrano reduced the plant height without affecting the number of leaves, this rootstock made the most compacted plants under well-watered conditions (denoted as the number of leaves per unit of plant height), especially compared to ungrafted Herminio (Fig. 3e). Water stress reduced the compactness in all plants except in those grafted onto Creonte.

3.3. Fruit yield, harvest index and agronomic water use efficiency

Although water stress reduced the total fruit yield by 10% in all graft combinations, rootstocks Atlante and Terrano (5%) and especially Creonte (15%) increased the total fruit yield under both conditions compared to non-grafted Herminio (Table 1). Water stress also reduced the commercial yield in all genotypes, although the three rootstocks increased it by 10–12% (Atlante and Terrano) and 20% (Creonte) under both irrigation regimes compared to the ungrafted Herminio plants. Indeed, the ungrafted Herminio plants registered the lowest number of commercial fruits and the higher incidence of sunscald–affected yield under both growing conditions (16–17%). This physiopathy registered a significant reduction due to the use of rootstocks, particularly under non-stress (8–12%), but also under water deficit (14% on average) (Table 1).

The harvest index increased under water deficit in all genotypes by 10–20%. Compared to the non-grafted Herminio, plants grafted onto Atlante (water stress) and Creonte (both conditions) registered between 7 and 15% increase in the harvest index (Fig. 4a). Indeed, Creonte increased WUEyld (commercial and non-commercial) by 16 and 10% compared to ungrafted Herminio and grafted onto the other rootstocks under both irrigation regimes, respectively (Fig. 4b).

Considering the number of fruits/shoot biomass ratio as a reproductive/vegetative trait, Creonte is clearly a reproductive rootstock, while Atlante is a vegetative type, compared to the ungrafted Herminio (Fig. 4c). Terrano is an intermediate type that maintains a
high reproductive/vegetative ratio under water stress compared to ungrafted Herminio and Atlante.

### 3.4. Fruit quality parameters

Water stress reduced all the physical fruit quality parameters, with exception of firmness (Table 2). Only the percentage of dry matter in the fruit increased under stress.

Rootstock Creonte improved all those parameters respect to the ungrafted plants and other rootstocks and minimised the negative effect on fruit length. Creonte only reduced the percentage of dry matter under non-stress conditions. Terrano also improved the fruit length and ecuatorial pericarp size compared to ungrafted Herminio, while Atlante improved firmness but reduced dry matter percentage under both growing conditions (Table 2).

In general, water deficit generally increased the chemical fruit quality parameters, while the use of rootstocks decreased antioxidant capacity and vitamin C and total phenolic concentrations (Table 3). Indeed, the most reproductive Creonte registered the lowest values in those parameters. Only punctual positive effects...
Fig. 2. Growth: Shoot biomass (leaf FW + stem FW) (A), leaf FW (B), Stem FW (C), Root FW (D) of three rootstock/scion combinations (H-Atlante, H-Creonte; H-Terrano) and ungrafted 'Herminio' pepper plants growing under stress (S) and non-stress (NS) conditions at 205 days after transplanting. Different capital letters point to significant differences between water conditions and small letters show significant differences between the treatments according to LSD test (p < 0.05).

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conditions</th>
<th>Herminio</th>
<th>H-Atlante</th>
<th>H-Creonte</th>
<th>H-Terrano</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>S</td>
<td>105.16 ± 11.03 Ka</td>
<td>109.36 ± 16.13 Ka</td>
<td>123.70 ± 26.90 Kb</td>
<td>106.94 ± 7.19 Ka</td>
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<tr>
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<td>NS</td>
<td>135.45 ± 8.63bA</td>
<td>143.25 ± 18.30bA</td>
<td>179.60 ± 21.25bB</td>
<td>144.49 ± 15.02bA</td>
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<td>Length (mm)</td>
<td>S</td>
<td>74.24 ± 5.57Aa</td>
<td>87.24 ± 10.16Aa</td>
<td>94.67 ± 7.28Bb</td>
<td>90.40 ± 15.04bA</td>
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<td>NS</td>
<td>96.92 ± 10.01Ab</td>
<td>96.64 ± 7.74Ab</td>
<td>102.42 ± 12.14Ab</td>
<td>102.18 ± 14.06bB</td>
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<td>Width (mm)</td>
<td>S</td>
<td>71.46 ± 7.27Aa</td>
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<td>69.81 ± 8.15Aa</td>
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<td>NS</td>
<td>74.13 ± 9.33Ab</td>
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<td>89.49 ± 4.53bB</td>
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<td>Firmness (kg cm⁻²)</td>
<td>S</td>
<td>2.04 ± 0.20Ab</td>
<td>2.18 ± 0.19bA</td>
<td>2.26 ± 0.30bA</td>
<td>1.98 ± 0.33bA</td>
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<tr>
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<td>1.96 ± 0.58Ab</td>
<td>2.26 ± 0.30bA</td>
<td>2.36 ± 0.34bA</td>
<td>2.16 ± 0.25Aa</td>
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<td>Apical thickness (mm)</td>
<td>S</td>
<td>6.34 ± 1.45Aa</td>
<td>6.62 ± 0.93Aa</td>
<td>7.18 ± 0.69bA</td>
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<td>7.12 ± 1.38Ab</td>
<td>7.32 ± 1.13Ab</td>
<td>7.72 ± 0.98bB</td>
<td>7.37 ± 0.53bB</td>
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<td>Basal thickness (mm)</td>
<td>S</td>
<td>6.20 ± 1.01Aa</td>
<td>6.37 ± 0.40bAa</td>
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<td>Dry Matter (%)</td>
<td>S</td>
<td>6.81 ± 0.75bB</td>
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<td>6.63 ± 0.59bAa</td>
<td>6.28 ± 0.60aAa</td>
<td>6.34 ± 0.99aAa</td>
<td>6.09 ± 0.33aAa</td>
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Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conditions</th>
<th>Herminio</th>
<th>H-Atlante</th>
<th>H-Creonte</th>
<th>H-Terrano</th>
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<tr>
<td>Vitamin C (mg/100 g FW)</td>
<td>S</td>
<td>87.6 ± 1.98 bb</td>
<td>85.3 ± 1.76 bb</td>
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<td>84.5 ± 2.01 bb</td>
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<td>79.6 ± 0.98 ba</td>
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<td>Anti-oxidant capacity (mg AAEkg⁻¹ FW)</td>
<td>S</td>
<td>98.3 ± 1.74 Aa</td>
<td>97.5 ± 2.83 bB</td>
<td>90.5 ± 1.78 ab</td>
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<td>NS</td>
<td>88.9 ± 1.52 bA</td>
<td>85.1 ± 1.60 bA</td>
<td>80.1 ± 2.01 aA</td>
<td>81.6 ± 1.86 Aa</td>
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<td>Total phenolics (mg CAE kg⁻¹ FW)</td>
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<td>254.82 ± 8.62 cB</td>
<td>230.3 ± 6.47 bB</td>
<td>204.0 ± 5.61 aB</td>
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<td>229.22 ± 7.54 cA</td>
<td>211.6 ± 5.76 bA</td>
<td>190.0 ± 6.27 aA</td>
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<td>Titratable acidity (% Citric Acid)</td>
<td>S</td>
<td>9.1 ± 0.0 Ab</td>
<td>10.1 ± 2.0Bb</td>
<td>9.8 ± 2.0bB</td>
<td>8.5 ± 1.0aB</td>
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<td>9.0 ± 3.0Aa</td>
<td>9.1 ± 2.0aB</td>
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<td>Brix</td>
<td>S</td>
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<td>EC (mS/cm)</td>
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were observed in the titrable acidity (Atlante and Creonte under stress) and 'Brix (Atlante under non-stress). Interestingly, all the rootstocks provoked a consistent increase in the EC of the fruit extract under both conditions, with and additional effect of the water stress in plants grafted onto Atlante and Terrano. This effect could indicate a higher capacity of the rootstocks to absorb and accumulate nutrients in the aerial organs, compared to the ungrafted Herminio plants (Table 3).

4. Discussion

Desirable traits that should be assessed in rootstock breeding for favorable grafted phenotypes (as a result of rootstock genotype x scion genotype x environment interactions) include generative versus vegetative crop development, marketable fruit yield and quality in the early and late season, tolerance to biotic and abiotic stresses and efficiency in the use of soil, water and fertilizers. In the case of pepper, *Capsicum annuum* x *C. baccatum* hybrid rootstocks have been developed commercially and patented to provide multiple disease resistances and a good balance between generative and vegetative vigour for high yields (Hennart, 2014).

In this study, water stress reduced both total and marketable fruit yield by 10–15% in all graft combinations, but the use of rootstocks increased total (5–15%) and commercial (10–20%) fruit yield under both conditions compared to non-grafted Herminio (Table 1). However, the performance of different rootstocks on vegetative/reproductive growth, gas exchange and fruit quality was different. Creonte was the best performant rootstock improving total and marketable fruit yield (Table 1) and some physical fruit quality traits (fruit dimensions and firmness) in both control and particularly deficit irrigation regimes (Table 2), but it reduced some chemical fruits quality traits, probably due to a dilution effect of different compounds by the rootstock-induced fruit growth (Table 3). Moreover, this rootstock significantly increases photosynthesis-related parameters (Fig. 1), the harvest index and the agronomic water use efficiency (Fig. 4a, b), compared to ungrafted Herminio and other rootstocks. Although Creonte registered the highest root biomass under control conditions, this rootstock was the most affected under water stress in this parameter. However, Atlante had the major root system under both conditions, induced the greater leaf and stem biomasses and plant height but without a significant additional effect on fruit yield. Therefore, Atlante can be considered as a vegetative rootstock while Creonte is rather generative (high reproductive/vegetative ratio) type (Fig. 4c). Plants grafted onto Terrano were more compact and registered the highest intrinsic WUE under water deficit, but producing similar yield to Atlante and greater than the non-grafted Herminio in spite of the low A and gs values (Fig. 1a, b). These results suggest much lower water require-
In general, grafting promotes vegetative growth due to the larger and more vigorous root system of the rootstocks, which are often capable of absorbing water and nutrients much more efficiently than ungrafted plants (Lee et al., 2010). However, growth and yield promotion differ, depending on rootstock characteristics and the capacity to alter shoot physiology (Albacete et al., 2009, 2014; Pérez-Alfocea et al., 2010). Indeed, there is no inter-relationship between production and plant vigour provided by the rootstock in pepper (Doñas-Uclés et al., 2015), as it occurs in this study. In this regard, it is important to know how the rootstock affect shoot development since this factor will importantly influence plant productivity and resource use efficiency. Thus, the use of low vigour rootstocks derived from a Solanum lycopersicum × S. pinnellifolium RIL population reduced the water use and increased the agronomic WUE by 40% in a tomato elite variety through reducing the leaf biomass, compared with self-grafted plants (Cantero-Navarro et al., 2016).

The interest in rootstock effects on water relations arises primarily because of the potential that the breeding or selection of improved rootstocks offers for improving water use efficiency and drought tolerance of horticultural crops (Jones, 2012). In particular since the scion transpiration rate and its acclimation to water deficit are controlled genetically by the rootstock through different genetic architectures (Marguerit et al., 2012). Two different mechanisms of rootstock-mediated resistance have been proposed in apple (Tworkoski et al., 2016). While a dwarfing rootstock produces higher levels of ABA (that may regulate stomatal opening and improve short-term drought resistance), a more invigorating rootstock may be drought resistant in the longer-term due to development of a more extensive root system. At reduced gs carbon assimilation (A) was low but greater in trees on dwarfing than on invigorating rootstock, such as it happens in Terrano, which registered the highest WUE under stress (Fig. 1E). In tomato, the ethylene precursor 1-aminoacyclopropane-1-carboxylic acid (ACC) seems to be a major rootstock-mediated factor negatively regulating shoot biomass (Albacete et al., 2008, 2009; Cantero-Navarro et al., 2016) and favouring agronomic WUE. The role of different hormones in those processes are being studied in pepper.

Since the vigour-related traits of the rootstock can influence the water relations, it can be speculated that dwarfing rootstocks are more adequate than invigorating rootstocks under drought conditions. In this study, the dwarfing effect by Terrano does not seem to have any advantage in terms of drought tolerance respect to the other rootstocks since the shoot growth parameters were similar (except plant height) under control conditions and were more affected by stress than in the non-grafted plants. However, if the high compactness of the plants grafted onto Terrano is an indirect responsible for the higher intrinsic WUE (mainly due to reduced gs and E) and the subsequent effect on water use, or it is due to a direct effect of the stomata functioning, remains an open question.

On the other side, it could be expected that invigorating rootstocks can provide drought tolerance through extraction a greater volume of water from the soil (Tworkoski et al., 2016). It could be the case of Atlante, however the plants grafted onto this rootstock were the most affected in shoot biomass (Fig. 2A) in spite of having the highest root biomass under stress (Fig. 2D), but without any benefit on fruit yield respect to Creonte. However, a different priority in biomass reallocation between shoot and root seems to exist between Creonte and the other graft combinations. Creonte maintains a preference in biomass allocation to the aerial parts under water stress in detriment of the root, while the opposite seems to occur in the other plants (Fig. 2). This capacity to maintain sink activity in the aboveground vegetative and reproductive organs in detriment of the root could be an adaptive advantage leading to shoot growth and yield through regulating source-sink
relations (Albacete et al., 2014), and therefore, to increased generative/vegetative ratio under water stress.

Even though the differences found in root biomass between genotypes were not significant in most cases, we cannot preclude that those differences actually exist, since it was practically impossible to get out the whole root system from the soil. This idea is supported by the fact that clear differences in root system architecture exist, with rootstocks having much more thicker and longer adventitious roots than the non-grafted Herminio plants (data not shown). A more developed root system would help to explain more vigorous plants, particularly under water deficit, but it does not seem to occur in this study, since the root biomass was most affected in the most productive rootstock Creonte (Fig. 4d). However, the rootstocks could contribute to a better water and nutritional status of the scion, which could contribute to a higher fruit yield in the grafted plants and a lower incidence of physiological disorders such as the sunscald of fruits. The higher leaf water content (Fig. 3D) and EC in the fruits (Table 3) of the grafted plants under water-limited conditions support this idea.

Creonte is clearly a generative-type rootstock since it increased fruit yield by 15% on average (both fruit number and fruit weight were 15 and 25–30% greater in Creonte than in Herminio under control and water stress, respectively) and had the highest reproductive/vegetative ratio under control and water stress conditions (Fig. 4c). The reduction in this parameter under stress was due to a decrease in the number of fruits (25% less than well watered plants) since the number of leaves was not affected in this graft combination. However, the most important traits of this rootstock are related to the alteration of leaf physiology in the scion, since this graft combination registered the highest values and stability of the photosynthesis-related parameters, as previously observed under thermal stress (López-Márín et al., 2013). This generative type together with a more efficient and stable photosynthetic system could be responsible for the increased and stable fruit yield under standard and deficit irrigation regimes. The physiological basis of this rootstock-mediated effect is currently under investigation. Similarly, rootstock-mediated improved productivity under deficit irrigation or salinity has been related to the capacity to maintain water status and photosynthesis in pepper (Penella et al., 2014, 2016) and tomato (Nilsen et al., 2014).

The rootstock-mediated alleviation of salt stress effects on yield and photosynthetic activity in pepper was not explained in terms of protection of oxidative stress due to the induction of protective antioxidants mechanisms (Penella et al., 2016). It was concluded that a constitutive enhanced root system and leaf protection (through proline accumulation) were responsible for maintaining shoot and root growth and photosynthetic performance under salinity. An increase in nutrient (K, Ca, Mg) uptake was also related to a better osmotic adjustment under salinity (Penella et al., 2016). A better osmotic adjustment due to higher inorganic solute conductivity is indirectly supported by the increased electrical conductivity found in the fruits of the grafted plants, which responded positively to the water stress while the ungrafted Herminio plants did not (Table 3).

In conclusion, the vigorous vegetative rootstocks Atlante does not provide any benefit in terms of fruit yield or drought resistance, but rather invest much more resources in developing unfruitful vegetative structures in the scion variety. Creonte is a reproductive rootstock that provides increased net photosynthesis, stable leaf area and increased yields (due to both fruit number and weight) under both irrigation regimes without affecting the vegetative structure of the scion variety. Terrano is a dwarfing-reproductive rootstock that mainly reduces the plant height without affecting shoot biomass and increases the intrinsic WUE and the commercial fruit yield under both irrigation regimes, while retaining the key fruit quality features of the scion.

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